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Microstructure of the Testis and Semen Analysis in Wistar Rats after Treatment with Ethanol Leaf Extract of *Acanthus Montanus.*

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ABSTRACT

Acanthus montanus is a herbal remedy for inflammation and Pains. This work studied the structure of the testes and analysed semen parameters in 21 Wistar rats. Eight weeks old rats weighing 135-150g were used. The leaves were dried, ground and extracted using 70% alcohol. Animals were randomly divided into 3 groups of 7 rats each. The control group was given distilled water. Test groups were administered with ethanol leaf extract of *Acanthus montanus*, 200mg/kg body weight of rats and 500mg/kg body weight for low and high doses respectively. The administration was done orally once a day with orogastric tube for 14 days. At the end of administration, the animals, at euthanasia with diethyl ether were sacrificed. The testes were dissected out and preserved in 10% buffered formaldehyde. The cauda epididymis was removed and several semen parameters were assessed. Data obtained was subjected to Analysis of Variance (ANOVA) and Pos hoc test (Dunnet's multiple comparison test) for significant differences. Results obtained indicated decreased spermatogenic cells, decreased sperm motility and viability ($P<0.05$), increased percentage of head abnormality, reduced sperm count but no effect on semen PH and testicular weight. In conclusion Extract altered testicular microstructure and some semen parameters.

Keywords: Acanthus montanus leaf extract, testes, rats

INTRODUCTION

The knowledge of plant use both as food and as therapy for ailment is common to many cultures. This knowledge continues to grow and has built up the basic part of research medicine called herbal medicine[1]. Ancient men used plants to treat common ailments and even life threatening diseases[2]. Therapeutic and health enhancing agents have been derived from plants, some in a more refined form such as tablets, capsules, etc. Today many countries globally practice plant based healing[3]

Acanthus montanus also called Bears's breech or mountain thistle is a shrubby spreading perennial plant that is native to tropical western Africa, the Balkans, Romania, Greece and Eastern Mediterranean[4]. It is a member of the *Acanthaceae* family. In Nigeria it is called Ahon-ekun by the yorubas, In Ibo, agameru[4] and Mbara ekpe by the Efiks (Cross River State). The plant is also used to protect against the devil in purification and exorcism[5]. In Cameroon, it is used for pains[6]. There is an overall decline in fertility rate[7]. With a shift to the use of herbal remedies without a knowledge of their phytochemistry coupled with no specificity in the dose, it has become increasingly important to study the actions of these commonly consumed herbs considering that possibly one of the major causes of today's infertility and sterility could be the diverse range of chronic exposure of individuals to the use of herbs which are either taken orally as decoction or otherwise, particularly those with asymptomatic ailments without the individual being aware of its effect.

Acanthus montanus (Nees) T. Anderson is a member of the acantheceae family, also known as bear's breech, mountain Thistle or alligator plan[8]. *It* is used to maturate abscesses, cough treatment, chest pains, given to children an emetics. It used in Cameroon to treat pain and threatened abortion[9]. has antimicrobial activity[4] . The plant has anti-inflammatory, analgesic properties[10]. It is also used to alleviate urethral discharge, constipation, rheumatic pain and syphilis[11]. Studies indicate that leaves of *Acanthus montanus* are used to treat respiratory ailment such as cold and pneumonia[12,13]. Macerated leaves of *Acanthusmontanus* are used against backache[14], bronchitis and bacterial infection[15]. It is used in the treatment of hepatitis and was effective carbon tetrachloride induced liver damage[16] Also used against

cases of inflammation[17,14] menstrual irregularities and dysmenorrhoea[12]. On the other hand antifertility and fetotoxic effects of the aqueous extract was reported[18]. Studies indicate that the leaf has been used to treat cases of threatened abortion [18]. It's effectiveness against urinary tract infections, urethral pains, endometritis, cystitis, leucorrhoea has been reported[4]. The antidiabetic, immunostimulatory and hypo cholesterolemic activities have also been documented[19,17,20] *Acanthus montanus* leaf extract is useful against bronchitis, bacterial infection, hormonal imbalance[15].

The major phytochemical component isolated from *Acanthus montanus* are tanins, saponins, terpenoids, steroids, Flavonoids and alkaloids. Further analysis showed that leaf contains Proteins, Cardiac glycoside, Reducing sugar, Carbohydrate, metals[14]. Thus the presence of phytochemicals in this plant validates the use of different parts of the plant as a potent medicinal plant. Literature is few concerning its effect on the gonads hence this study is done to assess possible side effect of consumption of ethanolic leaf extract of *Acanthus montanus* on the histology of the testis and semen parameters.

MATERIALS AND METHODS

Preparation of Extract : *Acanthus montanus* was purchased from the river side of Barcocoa, 8 Miles, Calabar municipality, authenticated by a taxonomist, Mr Effa, Effa A. in the department of Botany University of Calabar, with a voucher number 356. Fresh leaves of were washed under running tap water, air dried and later ground into powder using an electric blender. The powder was soaked in 70% ethanol for 48 hours at room temperature and was stirred at intervals. After 48 hours, it was filtered a material with small pores and again using No1 whatmann filter paper of pore size 0.45micrometer and funnel. The filtrate was concentrated in a water bath by evaporation at 40^{o}c to complete dryness yielding 20.5g of dry mater as the extract.

Experimental Animals: Twenty-one (21) adult albino male Wistar rats weighing between 130 - 177g from the Faculty of Basic Medical Sciences Animal House, university of Calabar were used. They were kept to acclimatize for two weeks under standard temperature 25 - 27^{0C}. They were randomly assigned into three groups of seven rats each. The animals in control group,' A' received distilled water, the same volume as the extract given to the test animals. The low dose group, 'B' was tested with the extract, 100mg/kg body weight While the high dose group 'C' was tested with the extract, 500mg/kg weight of rats. Administration was done orally using orogastric tube, once a day for 14 days.

All procedures involving animals were done by approval of the ethical committee on the Use and Care of Animals in the Faculty of Basic Medical Sciences, University of Calabar. The work was carried out in line with their guidelines and that of the National Academy of Sciences guide. At the end of the experiment, within 24 hours, the animals in euthanasia were sacrificed using diethyl ether. The testes were dissected out and preserved in 10% buffered formaldehyde and thereafter processed for staining with haematoxylin and eosin staining methods. The cauda epididymis was removed, incised and semen prepared for analysis. Sperm count was done according to the reported method[21].The sperm viability test was determined using Eosin-Nigrosin staining technique[22]. Sperm head abnormality test was done according to Ekaluo[23] . Sperm motility and PH were done as reported[21].

Data obtained from epididymal semen pH, motility, viability, count, sperm head abnormalities and weight of testes were subjected to analysis of variance (ANOVA) test for significant difference at followed by Dunnet's multiple comparison test as post hoc test. Statistical significance was considered at $P<0.05$ and $P < 0.001$ while least significant difference (LSD) test was used to separate the means.

RESULTS

Morphologically, there was no abnormality observed. Histolologically the following were observed.

Control group: Animals in this group were given distilled water only. Histological section of the testes from this group showed normal histological features with well defined layers. Numerous seminiferous tubules(ST) with intact basement membrane(BM) containing proliferating spermatogenic cells (SP) at various stages of maturation. The cell layers were thick with regular outline. The cells include spermatogonia A and B, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa within the lumen. Within the intervening interstitium were leydig cells (LE) in loose cluster. (Figure 1).

200mg/kgw group: Sections of the testes showed congested blood and cluster of leydig cells. some of the tubules in this group had lumen with reduced spermatozoa compared to the control group which had lumen densely filled with spermatogenic cells.

500mg/kg body weight group: Sections showed widely spaced seminiferous tubules with intact basement membrane containing proliferating germ cells at various stages of maturation. Tubules in this group had more empty lumen compared with the control group.

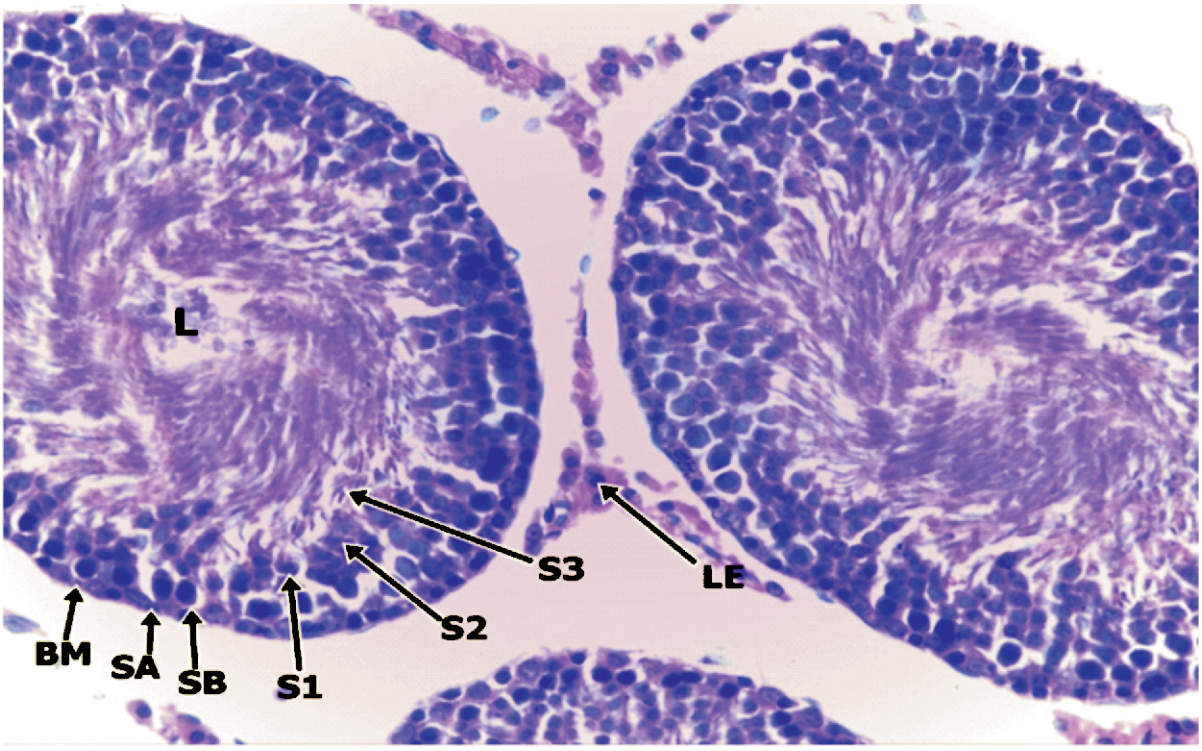

Figure 1: Section of the testis of rat from Control group. Mag. X400. Histological section of the Testis from this group shows normal histological features with well define layers from external to internal which include; numerous seminiferous tubules with intact basement membrane(BM), proliferating spermatogenic cells- spermatogonia A&B, Primary spematocyte, secondary spermatocyte, spermatids and matured spermatogonia at the centre of the lumen. (SA, SB, S_1, S_2, S_3). Leydig cells (LE) are found within the insterstitium.

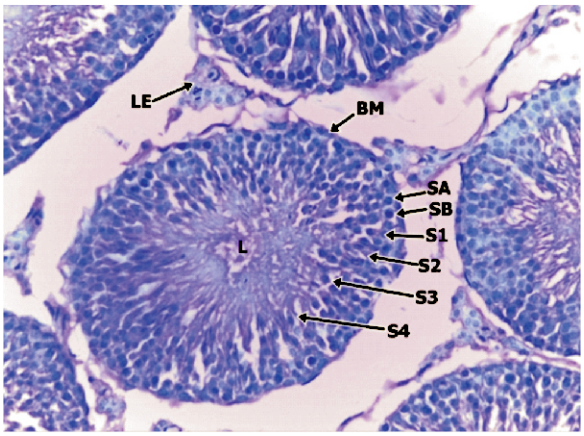

Figure 2: Section of the testis of rat from animals tested with extract, 100mg/kgw for 14days. Section showed loss of spermatogenic cells indicated by the presence of some spaces (SP) within the spermatogenic series of cells.

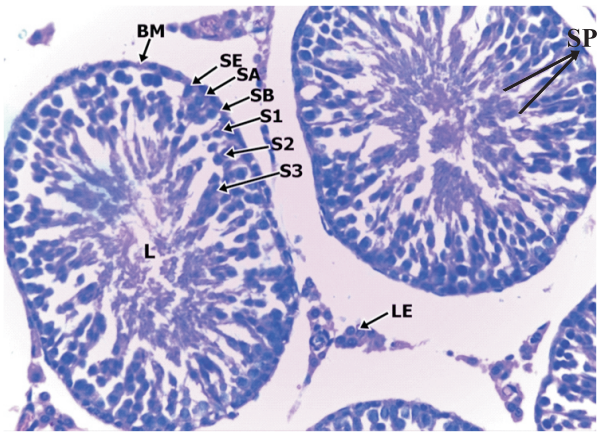

Figure 3: Sections of testis from test rats tested with extract, 500mg/kgw. Section showed increased spaces (SP) within the spermatogenic series of cells, indicating loss of germ cells and detachment from the basement membrane(BM). Reduced matured spermatozoa in the lumen(L). LE- Leydig cells.

Semen Analysis

The sperm motility: This decreased significantly in the experimental groups when compared with control group. Low dose was significantly different from control at $p<0.001$ while high dose was significantly different from low dose at $p<0.001$ (Figure 4).

Semen pH: This had no significant difference between the control and experimental groups (Figure 5).

The sperm viability: This was significantly reduced in the experimental groups compared to the control group. Sperm viability in low dose was significantly different from that in control at p<0.001, while the viability in high dose was significantly different from that in low dose at p<0.01 ((Figure 6).

Sperm head abnormality: This increased significantly in the experimental group compared to the control group. Values from test animals were significantly different from control at p<0.001. (Figure 7).

Sperm counts: This significantly reduced in the experimental groups compared to the control group. Low dose was significantly different from control at p<0.001 and high dose was significantly different from low dose at p<0.01 (Figure 8).

Testicular weight: This was taken in relation to the body weight. There was a dose dependent change in testicular weight. The weight in the 100/kg group increased while the weight decreased in the 500mg/kg group compared with the 100mg/kg group. weight (*P*<0.05) (Figure 9). Thus in summary the observations made from the semen analysis are: extract of *Acanthus montanus* caused a dose dependent histopathological changes in the testes of test animals. It reduced sperm count, decreased sperm motility(*P* <0.001) between the groups, reduced semen viability, increased percentage of sperm head abnormality, caused changes in testicular weight between groups (P < .05)and had no significant effect on semen pH.

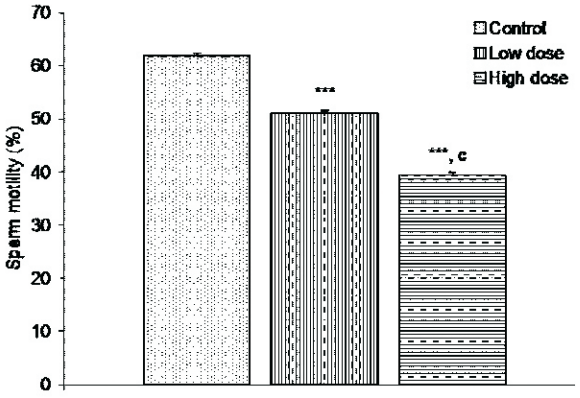

Figure 4: Sperm Motility in the Control and Test Groups of Rats after Administration of Ethanolic Extracts of *Acanthus montanus*. Values are expressed as mean ± SEM, n=7. ***=significantly different from control at p<0.001; C= significantly different from low dose at p<0.001

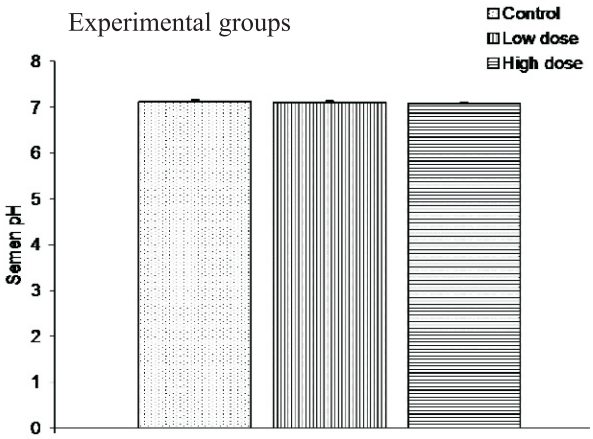

Figure 5: Chart showing Semen pH Between the Control Group and Test Groups of Rats after Administration of Ethanolic Extracts of Acanthus montanus. Values are expressed as mean ± SEM, n=7. No significant difference among groups

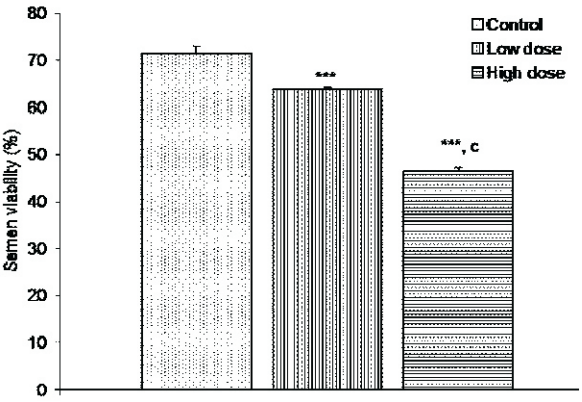

Figure 6: Chart Showing Sperm Viability Between the Control Group and Test Groups of Rats after Administration of Ethanolic Extracts of Acanthus montanus. Experimental groups**.** Values are expressed as mean ± SEM, n=7**.** ***=significantly different from control at p<0.001; C= significantly different from low dose at p<0.001

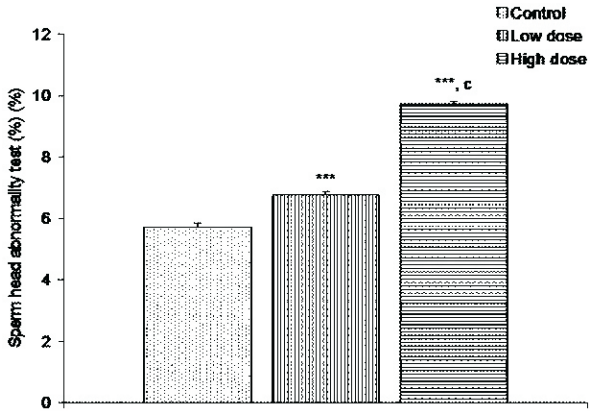

Figure 7: Sperm Head Abnormality Test (SHAT) Between the Control Group and Test Groups of Rats. Experimental groups. Values are expressed as mean ± SEM, n=7. ***=significantly different from control at p<0.001; C= significantly different from low dose at p<0.001

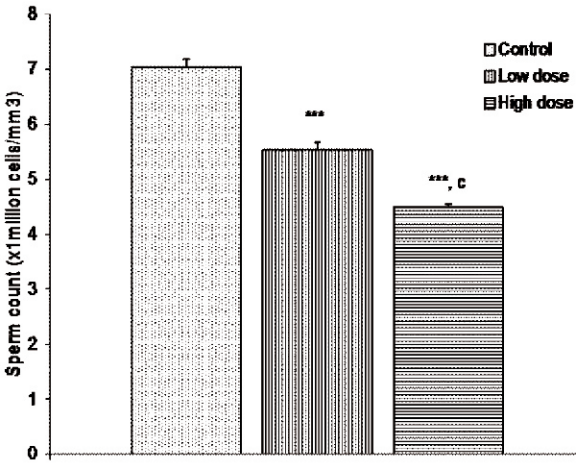

Figure 8: Comparison of sperm count between control group and test group of rats after administration of ethanolic extracts of Acanthus montanus. Experimental groups. Values are expressed as mean ± SEM, n=7. ***=significantly different from control at p<0.001; C= significantly different from low dose at p<0.001.

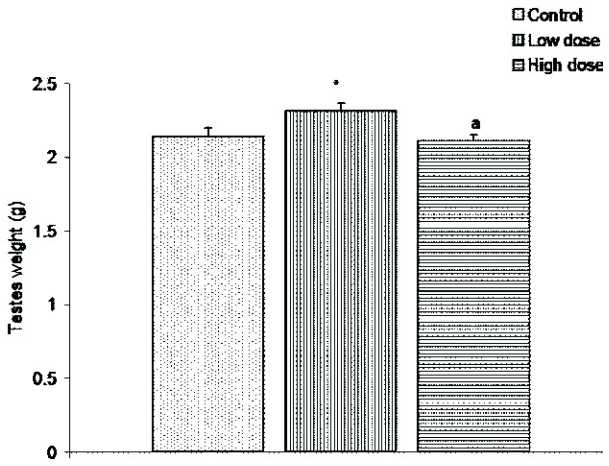

Figure 9: Testicular Weight (g/kg body weight) in the Control and Test Groups of Rats after Administration of Ethanolic Extracts of Acanthus montanus. Experimental groups. Values are expressed as mean ± SEM, n=7. *=significantly different from control at p<0.05; a= significantly different from low dose at p<0.05

DISCUSSION

Medicinal plants play a great role in human health as a result of its numerous phytochemical constituents whose therapeutic properties are employed in the management of various ailments and production of modern drugs[24]. The observed dose dependent changes observed in the testicular histology and semen analysis in the study could be attributed to its phytochemical constituents and the duration of administration.

Acanthus montanus contains, glycosides, steroids, triterpenes, saponins flavonoids[17] reducing sugar, terpenoids, acidic compounds, carbohydrate[8]. Chronic consumption can lead to an elevated levels of these phyochemicals in the plasma which may result in untoward effect. Elevated levels of sugar may lower testosterone. One method how sugar lowers testosterone is its effect on the adrenal glands. Sugar taxes the adrenal glands and these glands interrelate with the sex hormone glands (testes and ovaries) that produce testosterone and estrogen (Reference). Cardiac glycoside may alter testosterone secretion if in excess[25]. Testosterone is necessary for maintenance of seminiferous epithelium and spermatogenesis. The chronic consumption could possibly lead to testosterone alteration which in turn would have caused the loss of germ cells and detachment from the basement membrane in the group administered with extract, 500mg/kg weight.

In chronic consumption, steroids may interfere with the reproductive hormones. Reproductive toxins which have structure similar to endogenous hormones or growth factors or other compounds involved in reproduction could easily cause harm. As a result of their chemical activity, they can alkylate or chelate. Their metabolites also could exert toxic effects by inhibiting or inducing an enzyme[26]. The alterations in the semen parameters except semen PH suggests that extract may present adverse effect at high doses and should be used with caution. No proper scientific explanations can be given for this observations in this study except that the constituents may have interfered with the process of spermatogenesis at one stage or another resulting in abnormal structure and functions.

A study of the aqueous leaf extract o*f Acauthus montanus* on spermatogenesis in Swiss mice reported on an enhancing effect on spermatogenesis[27]. They observed adverse effect at a dose of 1000mg/kg weight. Their result is slightly different from ours but they used a different specie of animal and a different dose.

In conclusion administration of the ethanol leaf extract of *Acanthus montanus* causes loss of spermatogenic cells, decreased sperm count, motility, viability, increased sperm head abnormality but has no significant effect on semen PH.

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